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Research Article

Uncovering the hidden diversity of non-biting midges (Diptera, Chironomidae) from central Namibia, using morphology and DNA barcodes

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Abstract

The first study results on the Chironomidae fauna of central Namibia (Khomas, Otjozondjupa and Hardap regions) are presented, based on morphology and DNA-barcoding. The preliminary investigation led to the discovery of a new species *Paraphaenocladius namibiae* **sp. nov.** (Chironomidae, Orthocladiinae) and 17 new country records for Namibia.

Key words: Biodiversity, DNA barcoding, first records, integrative taxonomy, new species

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Introduction

Non-biting midges (Chironomidae) are an integral part of aquatic and terrestrial ecosystems across the world. They perform vital ecosystem functions, including the connection of wetlands with their surrounding areas via the transfer of matter and energy (Gratton et al. 2008). This function is especially important in arid landscapes, where there are few other sources of organic matter (Zinchenko et al. 2014). Namibia is a very arid country and this poses a major challenge to the country's economy (Reid et al. 2007). Therefore, studying the wetlands' contribution to the productivity and functioning of ecosystems in Namibia is of utmost importance for the management of all other ecosystems in the region. In 2018, on the initiative of the NCRST (National Commission on Research, Science, and Technology of Namibia) a collective collection permit was issued to the Participants of the 9th International Congress of Dipterology, held in Windhoek. The aim of this permit and research project was to support and improve the knowledge of the Namibian dipteran fauna for the benefit of basic- and applied research, and for Namibia itself.

We present the first results of our investigation on the Chironomidae fauna of central Namibia (Khomas, Otjozondjupa and Hardap regions). These are based on morphological and molecular (using DNA barcoding) assessments of the material collected in 2018. This preliminary investigation has led to the discovery of a previously unknown *Paraphaenocladius* (Chironomidae, Orthocladiinae), *P. namibiae* sp. nov., and 17 new country records for Namibia.

Materials and methods

Collection sites

Specimens were collected from the Khomas, Otjozondjupa and Hardap regions of central Namibia between 27 November – 8 December 2018, under the collective research permit issued by NCRST (authorization number AN20181007).

Specimens were collected by either sweep netting the vegetation, collecting exuviae and larvae with aquatic hand nets, Malaise traps, or light traps (see Suppl. material 1 for the details) (Figs 1A–D, 2A–D) All insect material was then exported to Germany for processing under an export permit issued by the Ministry of Environment and Tourism of Namibia (Number 119666). The material is now deposited in the natural history collection of the National Museum of Namibia in Windhoek (NMNW) and the College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China (SHOU). BOLD TaxonID Tree is available as Suppl. material 2.

Morphological identification and material handling

We are following the morphological terminology of Sæther (1980). Specimens were mounted in Hydromatrix (Micro Tech Lab GmbH) and Euparal, as per standard procedures for mounting small Diptera (Kirk-Spriggs 2017). Specimens were then identified using the following keys: Freeman 1953, 1954a, b, c, 1955a, b, 1956, 1957, 1958; Lehmnann 1979, 1981; Ekrem 2001; Ekrem et al. 2017; Gilka 2009; Andersen and Mendes 2010; Ferrington and Sæther 2011).

All slides were imaged using a Keyence VHX-6000 digital microscope, either using ring light- or cross-polarized coaxial illumination (Haug et al. 2008). The resulting images consist of vertical stacks and horizontal composites done with inbuilt microscope software (in case of VHX-6000) or PHOTOSHOP ELEMENTS CS 11 panorama functionality and PICOLAY open software (http://www.picolay.de).

DNA barcoding

Part of the sample was barcoded, with vouchers recovered afterwards. In the laboratory, we transferred the specimens to 96-well plates for processing via DNA barcoding. Lysis was performed at 56 °C for two hours only to ensure that all specimens remain undamaged. Then, we extracted the DNA using the NucleoSpin 96 Tissue Core Kit following the manufacturer's guidelines. We amplified the COI barcode region using the standard barcoding primers LepF1 and LepR1 (Ivanova and Grainger 2007).

The cleaned-up PCR products were sent to the LMU Sequencing Service at Biozentrum (Martinsried, Germany) for Sanger sequencing. Every specimen's COI barcode was sequenced as a forward and reverse strand. The traces were edited in BIOEDIT (Hall 1999), and a consensus sequence of the forward and reverse strands was obtained to be uploaded as a barcode to Barcode of Life Data System (BOLD, http://www.boldsystems.org/) (Ratnasingham and Hebert 2007). The original traces were uploaded as well. In addition, genomic DNA of a few specimens was extracted from the thorax or skin of larvae using Qiagen DNA Blood and Tissue Kit at the Shanghai Ocean University, Shanghai, China.

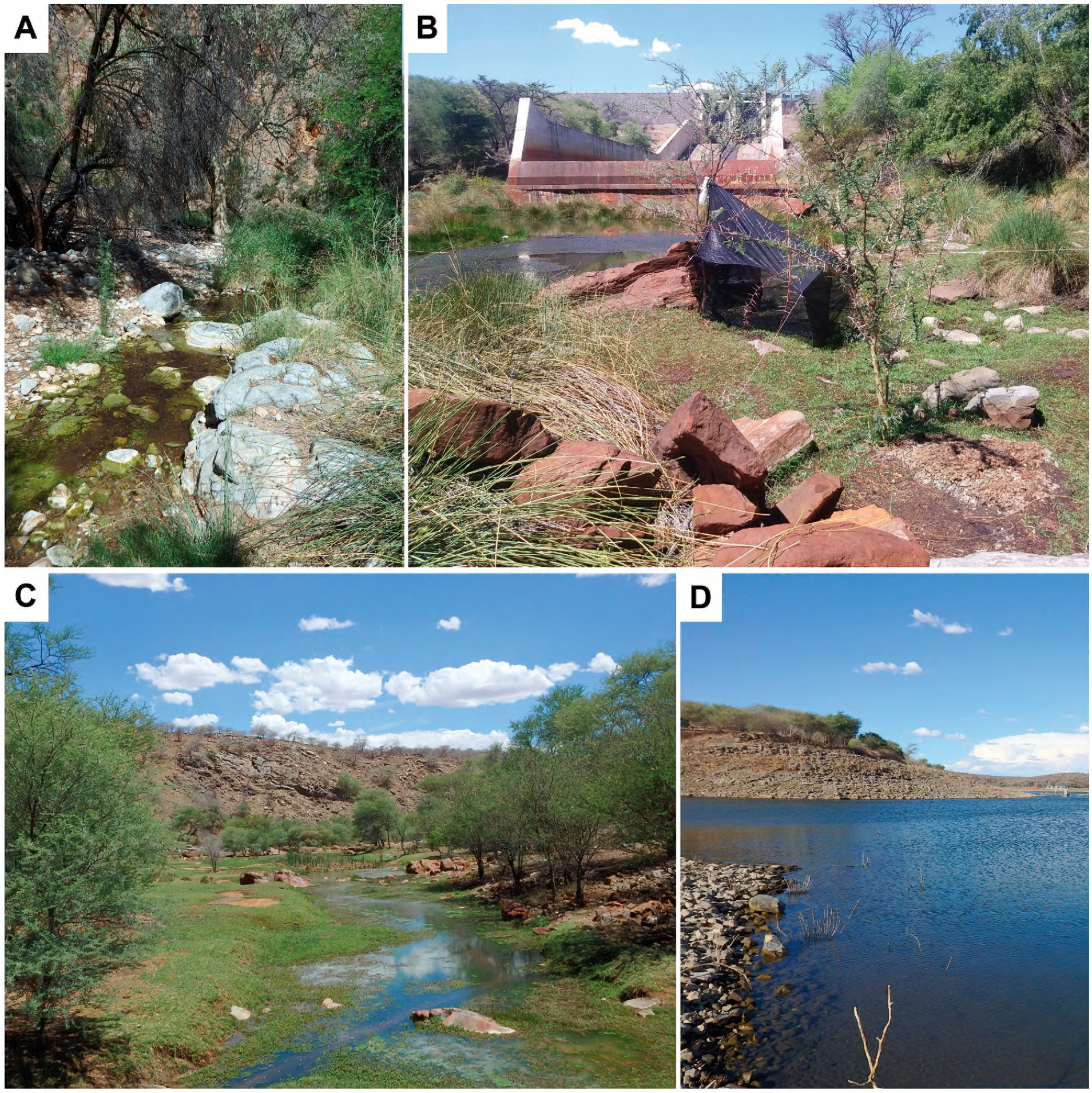

Figure 1. A–D photographs of the collection sites involved in this study **A** Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018 **B** Malaise trap deployed at Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2 Dec. 2018; facing towards the dam **C** Von Bach Dam Nature Reserve, Swakop river outflow, river valley; 22°0'53.28"S, 16°57'12.24"E; 2 Dec. 2018; facing away the dam **D** Von Bach Dam Nature Reserve, immediately above the dam; 2 Dec. 2018; 22°0'53.28"S, 16°57'12.24"E.

PCR amplifications of DNA barcodes, with the universal primers LCO1490 and HCO2198 (Folmer et al. 1994), were performed following the protocol from Lin et al. (2018). Sanger sequencing of the purified PCR products was conducted on the ABI 3730 at the BGI Genomic group (Beijing, China). Besides this, we also sent tissue samples of several specimens to the Canadian Centre for DNA Barcoding (CCDB, University of Guelph, Canada) using standard high throughput protocols (Hebert et al. 2018) to generate COI barcodes. In total, the 116 specimens successfully sequenced were added to the project on the Chironomidae of Namibia (http://dx.doi.org/10.5883/DS-NAMCHIR).

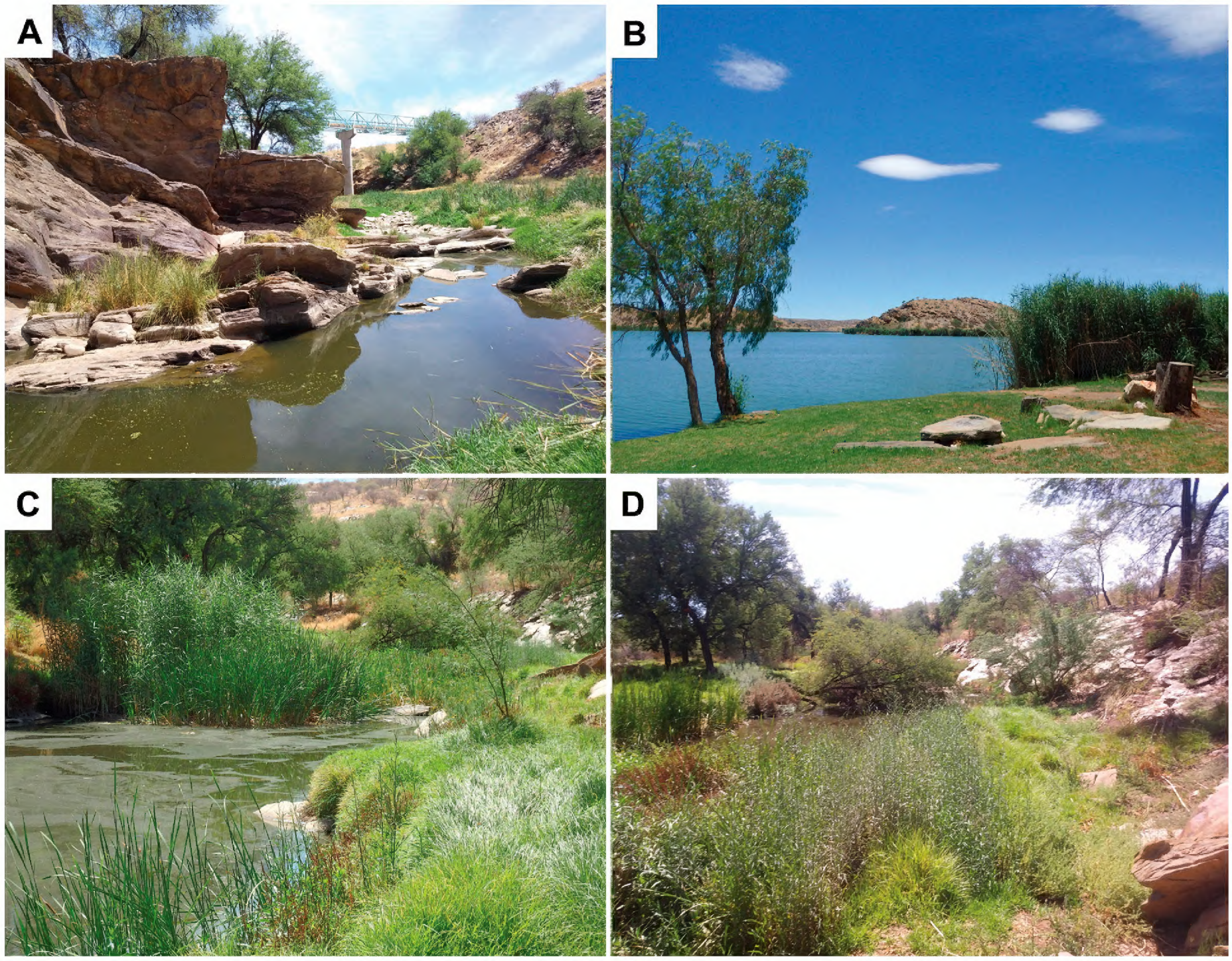

Figure 2. A–D photographs of the collection sites involved in this study **A** Windhoek, Arebbusch river22°34'28.92"S, 17°3'15.84"E; 3 Dec. 2018 **B** Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S, 17°0'3.6"E; 3 Dec. 2018 **C** Windhoek, Arebbusch river; 22°34'28.92"S, 17°3'15.84"E; 3 Dec. 2018 **D** Windhoek, Arebbusch river; 22°34'28.92"S, 17°3'15.84"E.

Results

Examination of the material has yielded 23 species and morphotypes of Chironomidae, with one of them being undescribed, and 17 new country-level records for Namibia.

Tanypodinae Thienemann & Zavřel, 1916

Tanypus guttatipennis Goetghebuer, 1935

Fig. 3A, B

Material examined. Namibia • 1♂; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018; G. M. Kwifte leg.; sweep net; NMNW (Fig. 1A).

Distribution. This is the first record of this species from Namibia, the species is otherwise widely distributed in the Afrotropical Region with records from Uganda, Democratic Republic of the Congo and South Africa (Freeman 1961; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2009). No specimens of this species were successfully barcoded as a part of this project.

Figure 3. *Tanypus guttatipennis*, male **A** wing **B** hypopygium.

Orthocladiinae Lenz, 1921

***Bryophaenocladius cristatus* Wang, Sæther & Andersen, 2002**
Fig. 4A–C

Material examined. Namibia • 1♂; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW (Fig. 1B–D). No specimens of this species were successfully barcoded as a part of this project.

Distribution. This is the first record of this species from Namibia, and outside of the species' type location in Ghana (Wang et al. 2002; Harrison 2004; Ashe and O'Connor 2012).

***Corynoneura dewulfi* Goetghebuer, 1935**

Material examined. Namibia • 5♂1♀; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018; G.M. Kwifte leg.; sweep net; NMNW.

No specimens of this species were successfully barcoded as a part of this project.

Distribution. This is the first record of the species from Namibia (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2012). The species is otherwise known from D.R. Congo, Ethiopia, South Africa, Tanzania, Uganda and Zimbabwe (Ashe and O'Connor 2012).

***Cricotopus flavozonatus* Freeman, 1953a**
Fig. 4D, E

Material examined. Namibia • several hundred ♂♀; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1–5 Dec. 2018; G. M. Kvifte/V. Baranov/X. Lin leg.; sweep net; NMNW; SHOU; BOLD specimen code: NAM14, NAM04, NAM13, NAM12; BOLD sequence ID: NAMCH024-20, NAMCH023-20, NAMCH017-20, NAMCH006-19; BOLD BIN: BOLD:ACG906 •1♂ 2♀; KHOMAS; Windhoek; 22°36'43.2"S, 17°5'27.6"E; 1 Dec. 2018; G. M. Kvifte/V.

Baranov/X. Lin leg.; NMNW; BOLD specimen code: NAM-Chiro31, NAM-Chiro32; BOLD sequence ID: NAMOE031-22, NAMOE032-22; BOLD BIN: BOLD:ACG9062.

Distribution. This is the first record of the species from Namibia. *Cricotopus flavozonatus* was previously recorded from Ethiopia, Uganda, South Africa, and Zimbabwe (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2012).

Cricotopus obscurus Freeman, 1953

Material examined. Namibia • 3♂; KHOMAS; Windhoek, Arebbusch river; 22°34'28.92"S, 17°3'15.84"E; 7 Dec. 2018; G.M. Kvifte leg.; sweep net; NMNW; 119♂♀; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2 Dec. 2018; V. Baranov leg.; sweep net; NMNW • 115♂♀; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW • 1♂; OTJOZONDJUPA; Gross Barmen; 22°6'38.16"S, 16°44'42"E; 4 Dec. 2018; X. Lin leg.; sweep net; SHOU; No specimens of this species were successfully barcoded as a part of this project.

Distribution. Species is present in Namibia (Harrison 2004). Lesotho, South Africa, Zimbabwe, Senegal (uncertain) (Ashe and O'Connor 2012).

Cricotopus scottae Freeman, 1956

Fig. 4F

Material examined. Namibia • 1♂14♀; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S, 17°0'3.6"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM-Chiro7; BOLD sequence ID NAMOE007-22; BOLD BIN: BOLD:ADM9835 • 1♂1♀; OTJOZONDJUPA; Düsternbrook; 22°15'11.52"S, 16°54'1.44"E; 4 Dec. 2018; G.M. Kvifte leg.; sweep net; SHOU • 8♂1♀, 1 larva; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW; BOLD specimen code: NAM-Chiro13, NAM-Chiro12; BOLD sequence ID: NAMOE013-22, NAMOE012-22; BOLD BIN: BOLD:ADM9835, BOLD:ACL1477; both, males and females, were barcoded (Fig. 6F).

Distribution. Species is present in Namibia (Harrison 2004). Species is otherwise known from Chad, D.R. Congo, Ethiopia, Niger, Nigeria, South Africa, Zimbabwe (Ashe and O'Connor 2012).

Limnophyes sp.

Material examined. Namibia • 1♂; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S, 17°0'3.6"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM85; BOLD: sequence ID: NAMCH069-19; BOLD BIN: BOLD:ACK4381.

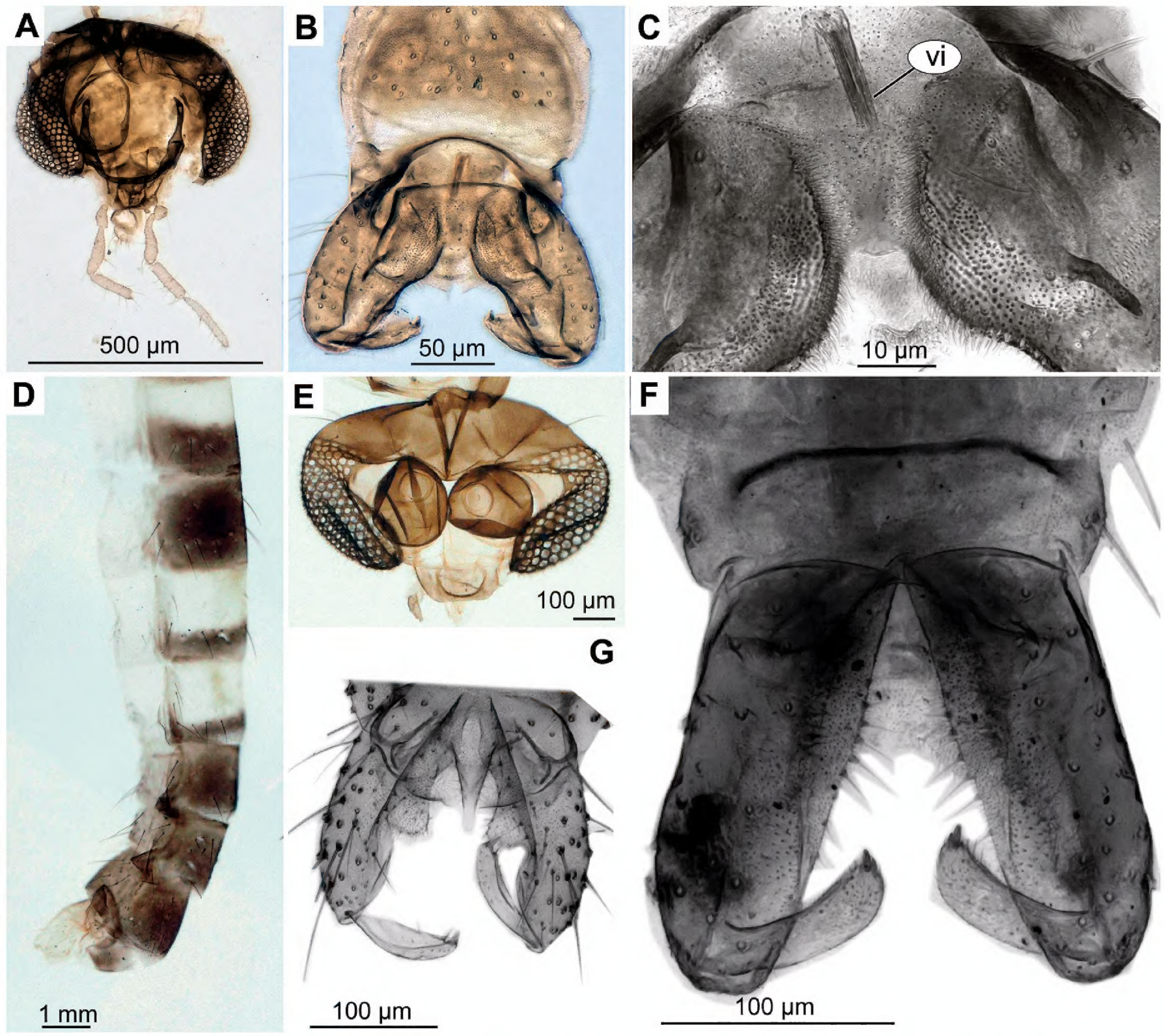

Figure 4. Orthocladiinae **A–C** *Bryophaenocladius cristatus* **D, E** *Cricotopus flavozonatus* **F** *Cricotopus scottae* **G** *Parametriocnemus scotti* **A, E** head, adult male **B, D, F, G** hypopygium **C** virga.

This is the first record of genus *Limnophyes* from Namibia (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2012).

***Parametriocnemus scotti* (Freeman, 1956)**
Fig. 4G

Material examined. Namibia • 8♂2♀, 1 larva; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018; G. M. Kvifte/V. Baranov, X. Lin leg.; Malaise trap; NMNW; BOLD specimen code: NAM37; NAM19; NAM-Chiro30; NAM-Chiro29; BOLD sequence ID NAMCH046-20; NAMOE030-22; NAMOE029-22; NAMCH007-19 BOLD:ADY1682.

Distribution. This is the first record of this species from Namibia (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2012). The species is otherwise known from Ethiopia, Kenya, Madagascar, South Africa, Uganda, Zambia and Zimbabwe (Ashe and O'Connor 2012).

Pseudosmittia unniae Ferrington & Sæther, 2011

Figs 5A–D, 6A–E

Material examined. Namibia • 1♂18♀; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S, 17°0'3.6"E; 3 Dec.2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code NAM86 BOLD sequence ID NAMCH072-20; NAMCH070-20 BOLD BIN BOLD:ADW9545; BOLD:ACK7891 • 2♂; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°31'44.4"S, 17°0'3.6"E; 2–10 Dec. 2018; Malaise trap; V. Baranov leg.; NMNW; BOLD specimen code: NAM81, NAM89, BOLD sequence ID NAMCH065-20, NAMCH072-20, BOLD BIN: BOLD:ADW9545 • 9♂1♀, OTJOZONDJUPA; Gross Barmen; 22°6'38.16"S, 16°44'42"E; 4 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code NAM61, NAM60, NAM59, NAM-Chiro51, NAM-Chiro50, NAM-Chiro49, NAM-Chiro48, NAM-Chiro45; BOLD sequence ID: NAMCH051-20, NAMCH050-20, NAMCH0549-20, NAMOE051-22, NAMOE050-22; NAMOE049-22, NAMOE048-22, NAMOE045-22, NAMOE044-22; BOLD BIN BOLD:AEG0717; BOLD:ADW9545.

Distribution. This is the first record of this species from Namibia (Ferrington and Sæther 2011; Ashe and O'Connor 2012). Comparison of current sequences with existing BINs on the BOLD system has shown the presence of this species also in South Africa.

Psectrocladius cf. *schlienzi* Wülker, 1956

Material examined. Namibia • 1♂; OTJOZONDJUPA; Gross Barmen; 22°6'38.16"S, 16°44'42"E; 4 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM65; BOLD sequence ID: NAMCH055-20; BOLD BIN: BOLD:ACK4896.

Distribution. This is the first record of the species from Namibia, but numerous representatives of the same BIN were previously recorded from South Africa (BOLD: ACK4896, Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2012). It is worth noting that this species is otherwise distributed in the Palaearctic (Austria, Denmark, Finland, Germany, United Kingdom, Italy, Moldova, Mongolia, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland) (Ashe and O'Connor 2012). It is, therefore, worth considering that the specimen that we have found belongs to a yet undescribed species of *Psectrocladius* related to *P. schlienzi*. More material is needed before we can test this hypothesis.

Paraphaenocladius Thienemann, 1924

Paraphaenocladius impensus (Walker, 1856)

Material examined. Namibia • 1♂; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S, 17°0'3.6"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM83; BOLD sequence ID: NAMCH067-20; BOLD BIN: BOLD:ACK2655; .

Distribution. This is the first record of the species from Namibia, but numerous representatives of the same BIN were previously recorded from South

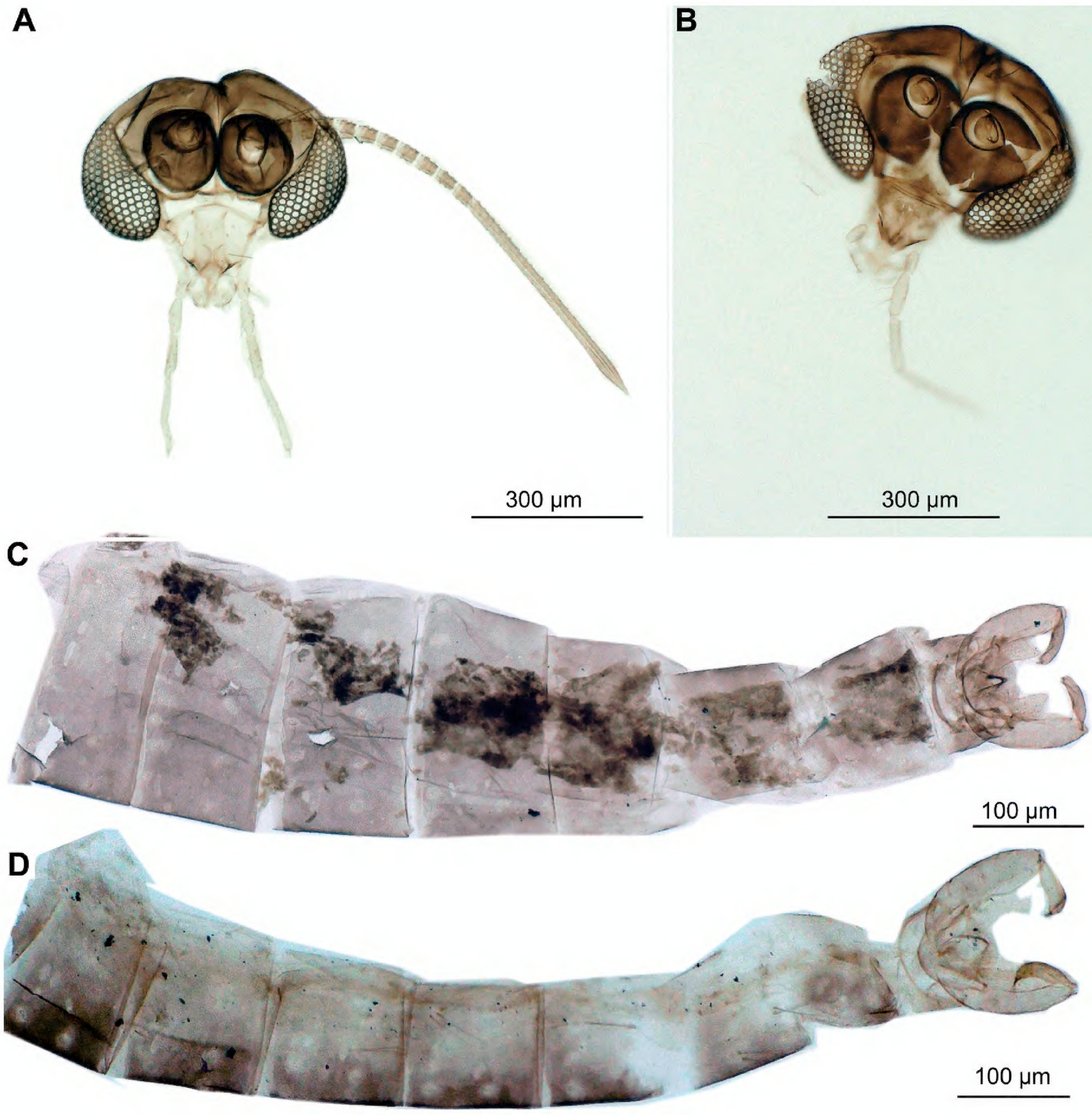

Figure 5. *Pseudosmittia unniae* adult male **A, B** heads **C, D** abdomens with hypopygium.

Africa (BOLD:ACK2655, Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Ashe and O'Connor 2012). The species is otherwise known from the multiple countries in the Palaearctic, Nearctic and Oriental regions. This is the first Afrotropical record (although the species is recorded from Palaearctic parts of the African continent, i.e. Algeria) (Ashe and O'Connor 2012).

***Paraphaenocladius namibiae* Baranov, sp. nov.**

https://zoobank.org/C91D58C6-2C92-4590-815E-AF68B47010E5

Figs 7A–C, 8A, B

Material examined. *Holotype*. Namibia • 1♂; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018; V. Baranov leg.; sweep net; Holotype is deposited at NMNW.

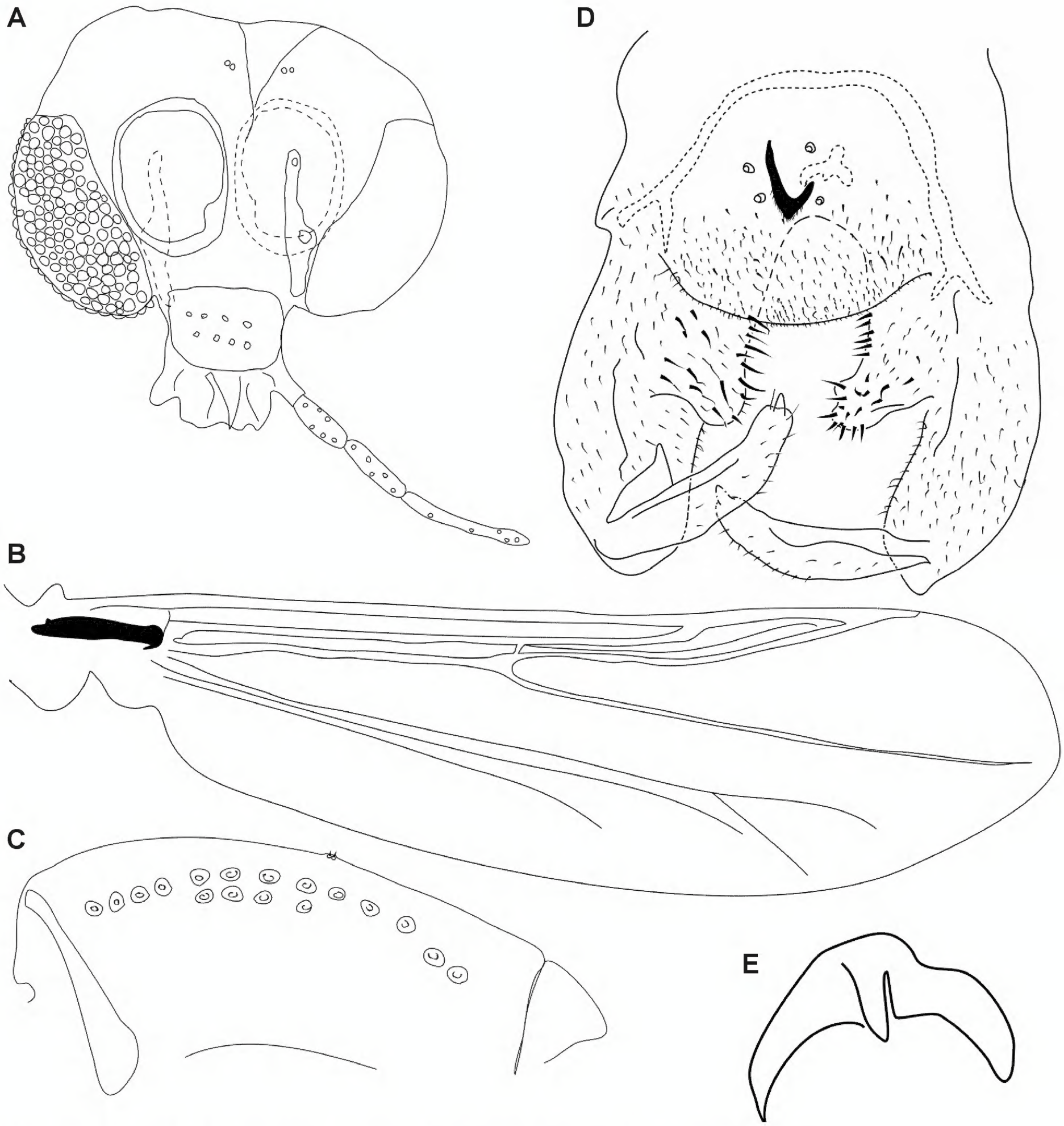

Figure 6. *Pseudosmittia unniae* adult male **A** Head **B** wing **C** scutum **D** hypopygium **E** virga.

Paratypes. Namibia • 2♀; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018; V. Baranov leg.; sweep net; NMNW; BOLD specimen codes: NAM-Chiro27; NAM-Chiro28; BOLD sequence ID:NAMOE027-22, NAMOE028-22. (poorly preserved, used in barcoding, vouchers non-recovered).

Both sequences were of lower quality, providing rather inconclusive barcode-based identification (See Suppl. material 1). The closest match in BOLD v.4 database was *Corynoneura* sp. (private record), with match of the barcode of 87.99% (Suppl. material 3).

Diagnosis. Differs from all other known species of *Paraphaenocladius* based on the combination of the cell proximal to crossvein *r–m* with no setae, anal point

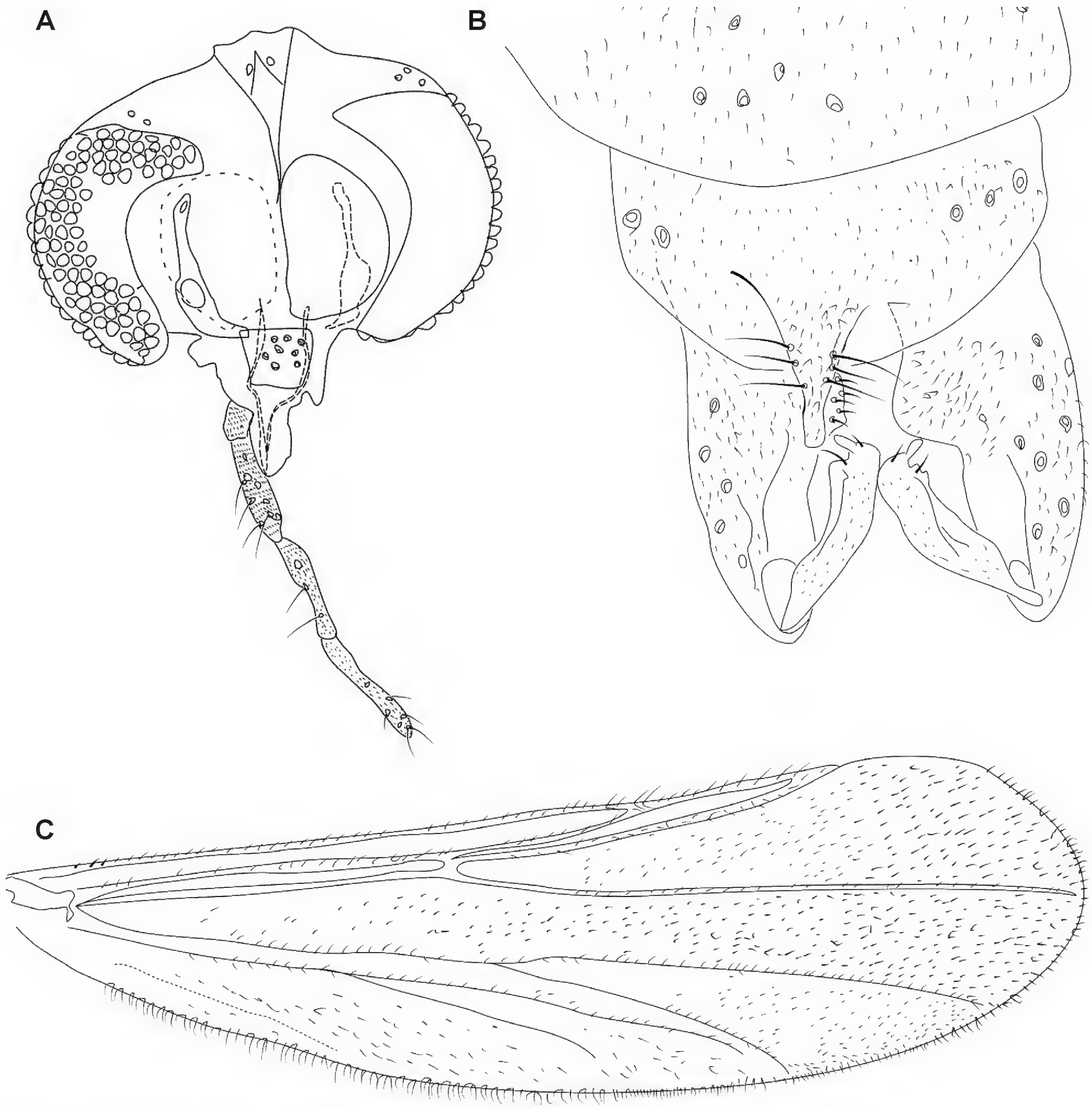

Figure 7. *Paraphaenocladius namibiae* sp. nov. holotype, adult male **A** head **B** hypopygium **C** wing.

of abdominal tergite X with parallel-sided tip, free of visible setae (except for a few microtrichia, virga absent, gonostylus with low, elongated crista dorsalis.

Description. Adult male (holotype, male; n=1).

Total length 1.9 mm, wing length 1.4 mm. Overall greenish colour, with yellow stripes on the scutum, small chironomid.

***Antennae*:** holotype was missing antennae upon sorting out from the samples.

***Head*:** Eyes with short, wedge-shaped extension. Temporal setae (n=1) 9, with 4 inner and 5 outer verticals, 3 orbital setae, clypeus with 8 setae. Tentorium 120 µm. Palpomeres' length in µm (n=1): 2^{nd} -33, 3^{rd} – 88, 4^{th} -95, 5^{th} -95 (Figs 7A, 8B).

***Thorax*:** Anteropronotal setae -3, Dorsocentral setae -15, Acrostichals -5, scutellars – 8.

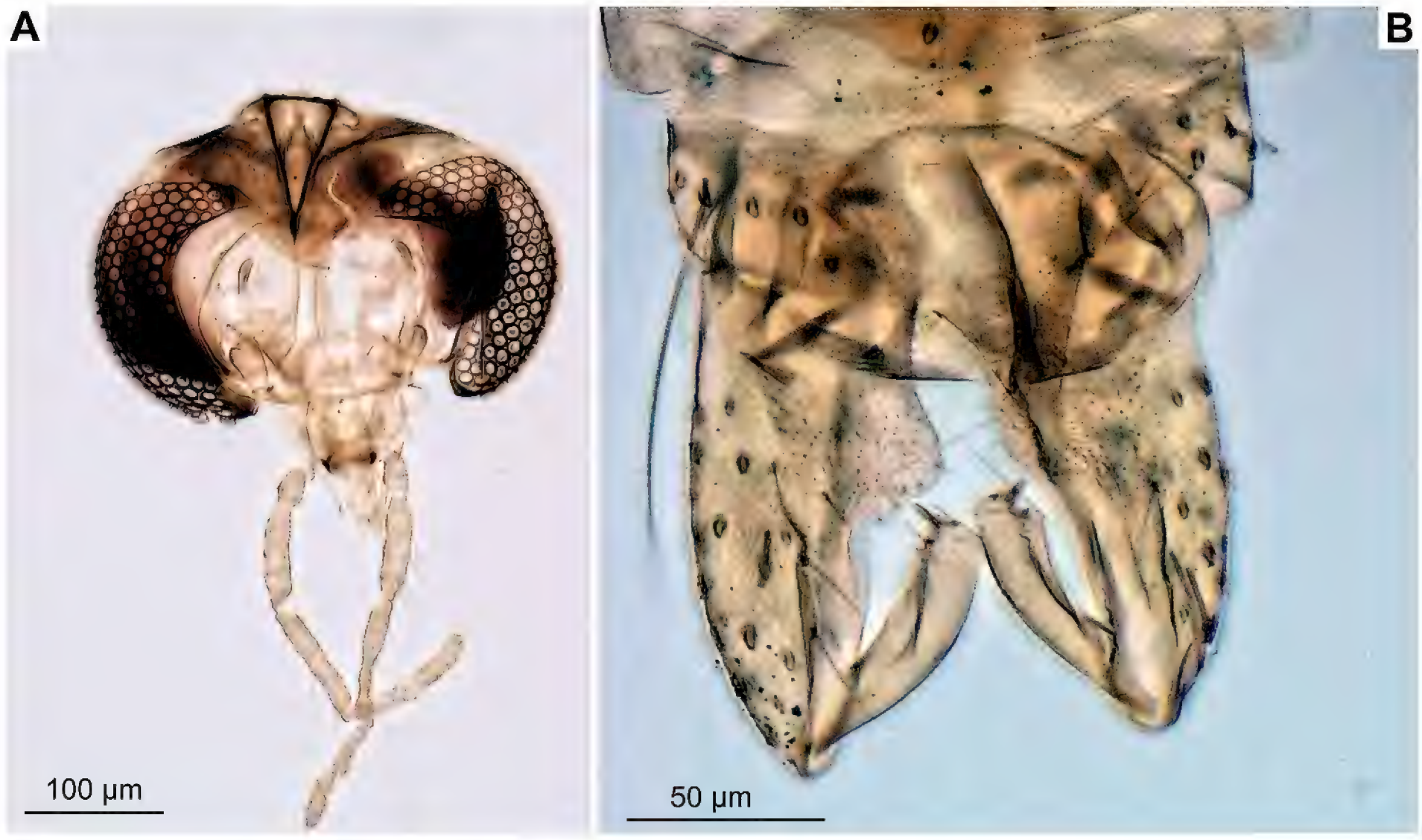

Figure 8. *Paraphaenocladius namibiae* sp.nov. holotype, adult male **A** head **B** hypogium

***Legs*:** all legs are missing tarsomeres. Fore and mid tibiae with one tibial spur, hind tibia with two spurs. Leg elements lengths as listed in Table 1.

Wing 1.4 mm long. Anal lobe strongly reduced. Costal extension 70 µm long, with 6 non-marginal setae. Cu_1 slightly sinuate. *R* with 28 setae. R_1 with 11 setae, R_{4+5} with 30 setae. *r–m* bare, *M* bare, M_{1+2} with 61 setae, M_{3+4} with 48 setae. *Cu* with 29 setae, Cu_1 with 33 setae. *CuP* with 27 setae (Fig. 7C).

***Hypopygium*.** Anal point with mostly bare apex (bearing some microtrichia), 12 µm long, 5 µm wide, tip parallel-sided. Anal point with three pairs of strong lateral setae at the base. Sternapodeme 65 µm long, phalapodeme 36 µm long. Virga absent. Gonocoxite 100 µm long, with large, rounded inferior volsella. Gonostylus 55 µm long. Megasetae 7 µm long. Gonostylus with a strong, apically rounded megasetae (Figs 7B, 8B).

Etymology. Named for Namibia, the species' country of origin.

Comments. Species was attributed to genus *Paraphaenocladius*, based on the combination of bare eyes with hairy wings, with Costal extension ending proximally to the tip of M_{3+4} and Cu_1 curved.

Based on the combination of the cell m proximal to crossvein *r–m* with zero setae, triangular anal point, with basal setae and bare apex, longer than wide and absent virga, the new species appears to belong to the *P. dewulfi* -species group sensu Sæther & Wang, 1995. This group consists of three previously described species of *Paraphaenocladius*, inhabiting the Afrotropics: *P. dewulfi* (Goetghebuer, 1936), *P. cuneipennis* (Freeman, 1961) and *P. crassicaudatus* Sæther & Wang, 1995.

Among the three, *P. namibiae* sp. nov. is most like *P. crassicaudatus*, due to the general similarity of the hypopygium morphology, most evident in the relatively broad anal point, strong crista dorsalis and broad inferior volsella.

Distribution. Species is only known from its type locality so far (Fig. 1A).

Table 1. Length (in µm) of leg segments of *Paraphaenocladius namibiae* sp. nov., male (n = 1).

Leg	Femora	Tibia	Ta1	Ta2	Ta3	Ta4	Ta5
Foreleg	300	250	–	–	–	–	–
Midleg	300	260	–	–	–	–	–
Hindleg	340	300	–	–	–	–	–

Chironominae Macquart, 1838

Tanytarsini Zavřel, 1916 [in Thienemann and Zavřel 1916]

Cladotanytarsus pseudomancus (Goetghebuer, 1934)

Fig. 9A, B

Material examined. Namibia • 500♂♀; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; Malaise trap; V. Baranov leg.; NMNW; BOLD specimen code: NAM-Chiro39 ; NAM-Chiro38; NAM-Chiro37; NAM-Chiro36; NAM-Chiro35: NAM50; NAM49; NAM51; NAM-Chiro41; NAM-Chiro40; BOLD sequence ID: NAMOE039-22; NAMOE038-22; NAMOE037-22; NAMOE036-22; NAMOE035-22; NAMCH013-19; NAMCH012-19; NAMCH048-20; NAMOE041-22; NAMOE040-22; BOLD BIN: BOLD:ACK2243 • 1♂; KHOMAS; Windhoek; 22°36'43.2"S, 17°5'27.6"E; 1 Dec. 2018; G. M. Kvifte, V. Baranov, X. Lin leg.; NMNW; BOLD specimen code: NAM-Chiro5; BOLD sequence ID: NAMOE005-22; BOLD BIN: BOLD:ACK2243 • 1♂; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S, 17°0'3.6"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM97; NAM93; BOLD sequence ID: NAMCH080-20; NAMCH076-20; BOLD BIN: BOLD:ACK2243 • 1 larva, 1pupa; OTJOZONDJUPA; Gross Barmen; 22°6'38.16"S, 16°44'42"E; 4 Dec. 2018; X. Lin leg.; sweep net; SHOU.

Distribution. This is the first record of the species from Namibia (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004). *Cladotanytarsus pseudomancus* is a widespread species, thus far recorded from the Afrotropical Region, including Madagascar, from the Oriental Region: India (West Bengal), China (Hainan) and the Palaearctic Region: Russian far east, France, Egypt, Saudi Arabia and Oman (Gilka 2009).

Tanytarsus pallidulus Freeman, 1954

Fig. 9C

Material examined. Namibia • 430♂♀; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; Malaise trap; V. Baranov leg.; NMNW • 1♂; KHOMAS, Windhoek, 22°36'43.2"S, 17°5'27.6"E; 8 Dec. 2018; G. M. Kvifte, V. Baranov, X. Lin leg.; NMNW; BOLD specimen code: NAM-Chiro6; BOLD sequence ID: NAMOE006-22; BOLD BIN: BOLD:AAH9824.

Distribution. This is the first record of the species from Namibia (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004). Otherwise, this species is occurring in South Africa, Mozambique, Nigeria, Zimbabwe, Democratic Republic of Congo and Saudi Arabia (Freeman 1956; Freeman and Cranston 1980; Cranston and Judd 1989; Ekrem 2001; Harrison 2004).

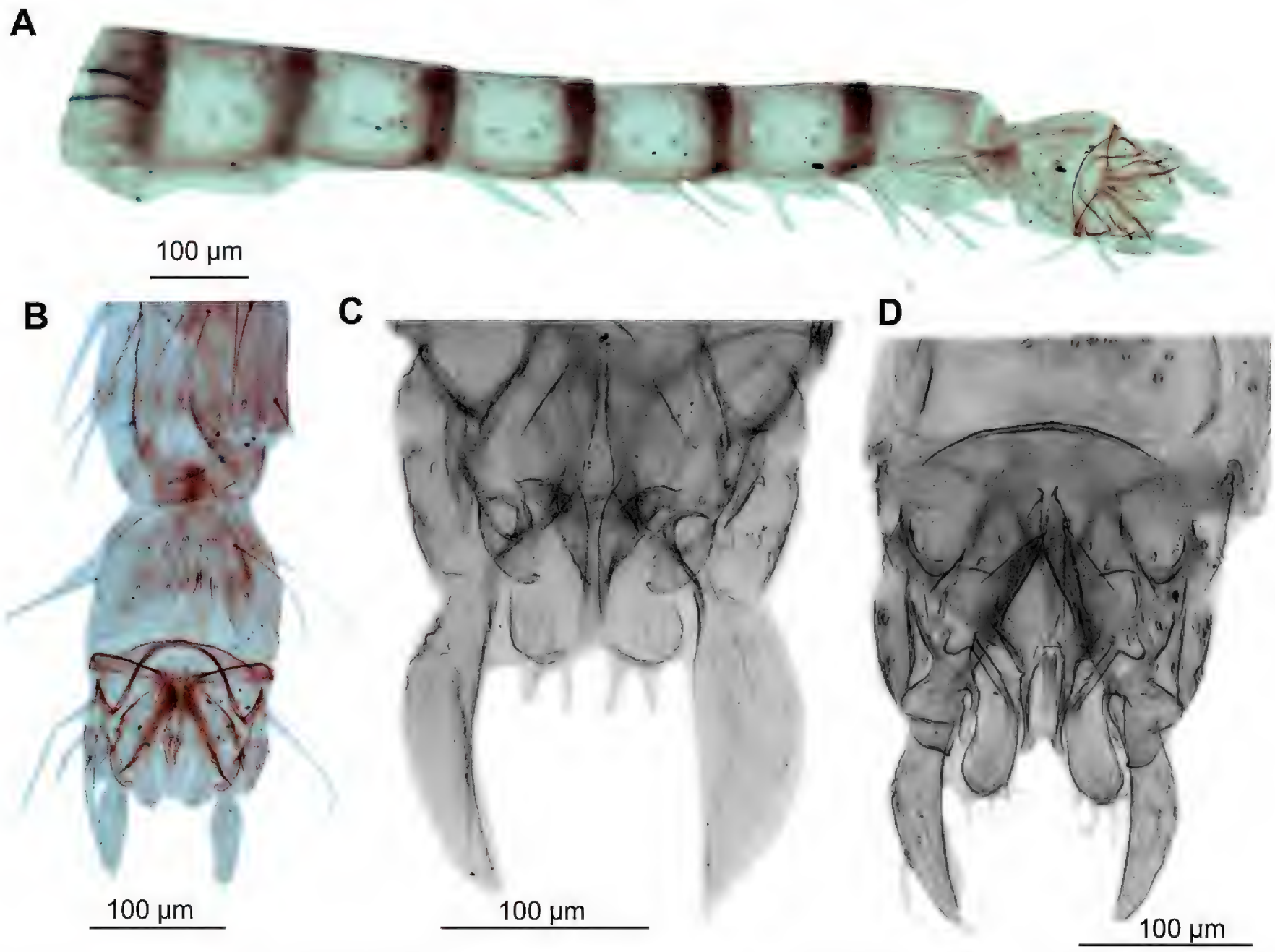

Figure 9. Tanytarsini, adult males, hypopygia **A, B** *Cladotanytarsus pseudomancus* **C** *Tanytarsus pallidulus* **D** *Tanytarsus bifurcus*.

***Tanytarsus bifurcus* Freeman, 1958**
Fig. 9D

Material examined. Namibia• 6♂; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow, 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; Malaise trap; V. Baranov leg.; NMNW• 1♂; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1–5 Dec. 2018; sweep net; G. M. Kvifte, V. Baranov, X. Lin leg.; SHOU; BOLD specimen code: NAM-Chiro19; BOLD sequence ID: NAMOE019-22; BOLD BIN: BOLD:ADU6503.

Distribution. This is the first record of the species from Namibia (Freeman and Cranston 1980; Curtis 1991; Harrison 2004). Otherwise this species is occurring in Burkina Faso, Chad, Mali, Nigeria and Ghana (Freeman 1958; Dejoux 1968a, 1968c, 1974; Freeman and Cranston 1980; Ekrem 2001).

Chironomini Macquart, 1838

***Chironomus transvaalensis* Kieffer, 1923**
Fig. 10A, B

Material examined. Namibia • 30♂24♀; KHOMAS; Windhoek, Arebbusch river; 22°34'28.92"S, 17°3'15.84"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM-Chiro3; BOLD sequence ID: NAMOE003-22 • 3♀;

KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S; 17°0'3.6"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM-Chiro23; NAM-Chiro54; NAM-Chiro3; BOLD sequence ID: NAMOE023-22; NAMOE054-22; NAMOE003-22; BOLD BIN: BOLD:AAW3995 • 1♀; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW; BOLD specimen code: NAM-Chiro34; BOLD sequence ID: NAMOE034-22; BOLD BIN: BOLD:AAW3995 • 1♂14♀; OTJOZONDJUPA; Düstenbrook; 22°15'11.52"S, 16°54'1.44"E; 4 Dec. 2018 ; G. M. Kvifte leg.; NMNW.

Distribution. The species is widely distributed in Namibia and the rest of the Afrotropics, as well as southern Palaearctic (Lindner 1976; Curtis 1991; Harrison 2004).

***Chironomus calipterus* Kieffer, 1908**

Fig. 10C

Material examined. Namibia • 67♂; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW.

Distribution: The species is widely distributed in Namibia and rest of the Afrotropics as well as the southern Palaearctic (Freeman 1957; Lindner 1976; Curtis 1991; Harrison 2004; Andersen and Mendes 2010).

***Dicrotendipes fusconotatus* (Kieffer, 1922)**

Material examined. Namibia • 3♂; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; 22°31'44.4"S; 17°0'3.6"E; 3 Dec. 2018; G.M. Kwifte leg.; sweep net; NMNW; BOLD specimen code: NAM-Chiro1; BOLD sequence ID: NAMOE001-22. •1♂; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S; 16°13'44.76"E; 1 Dec. 2018; sweep net; V. Baranov leg.; NMNW.

No specimens of this species were successfully barcoded as a part of this project.

Distribution. This is the first record of the species from Namibia; otherwise it occurs in Chad, Democratic Republic of the Congo, Egypt, Israel, Niger, Nigeria, Uganda, South Africa and Zimbabwe (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004).

***Dicrotendipes septemmaculatus* (Becker, 1908)**

Fig. 10D

Material examined. Namibia • 5♂, 2 larvae; 2 pupae; KHOMAS; Windhoek, Arebbusch river, Goreangab Reservoir; S22°52'90"S; 17°0'3.6"E; 3 Dec. 2018; G.M. Kwifte leg.; sweep net ; NMNW; BOLD specimen code: NAM-Chiro4; NAM88; NAM95; NAM94; NAM91; NAM90; BOLD sequence ID: NAMOE004-22; NAMCH016-19; NAMCH078-20; NAMCH077-20;

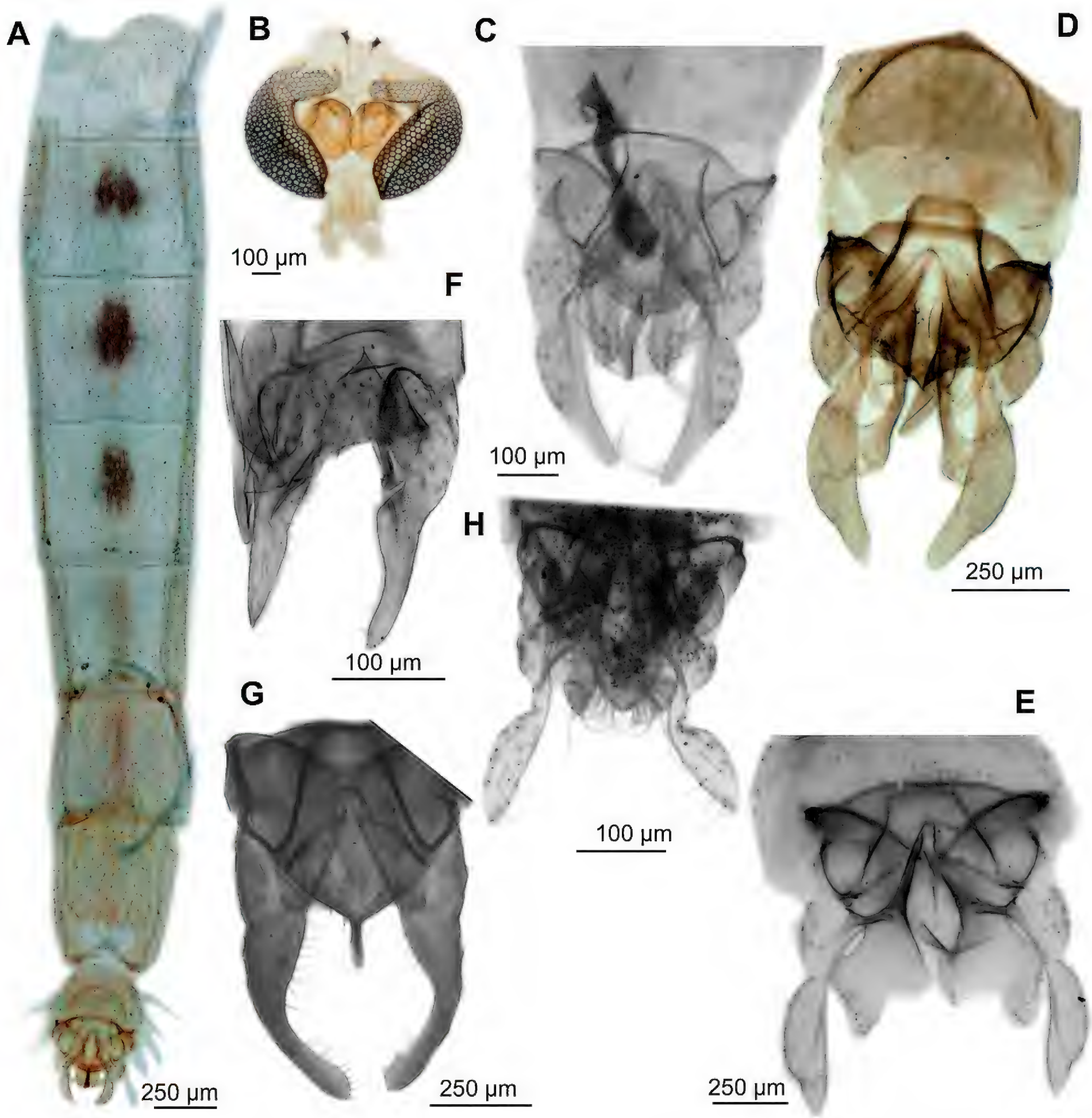

Figure 10. Chironomini, adult males **A, B** *Chironomus transvaalensis* **C** *Chironomus calipterus* **D** *Dicrotendipes septemmaculatus* **E** *Kiefferulus brevipalpis* **F** *Parachironomus acutus* **G** *Paracladopelma rhodesianus* **H** *Polypedilum abyssiniae* **A, C–H** hypopygia **B** head.

NAMCH074-20; NAMCH073-20; BOLD BIN: BOLD:ACK4391 • 1♂; KHOMAS; Windhoek; 22°36'43.2"S, 17°5'27.6"E; 3 Dec. 2018; X. Lin leg.; light trap; SHOU; BOLD specimen code: NAM76; BOLD sequence ID; NAMCH015-19; BOLD BIN: BOLD:ADY3994 • 1♀; HARDAP; Naukluft Mountain Zebra Park, Tufa waterfalls; 24°15'47.88"S, 16°13'44.76"E; 1 Dec. 2018; V. Baranov leg.; sweep net; NMNW; BOLD specimen code: NAM-Chiro25; BOLD sequence ID: NAMOE025-22; BOLD BIN: BOLD:ACK4391 • 1♀; OTJOZONDJUPA; Gross Barmen; 22°6'38.16"S, 16°44'42"E; 4 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM66; BOLD sequence ID: NAMCH056-20; BOLD BIN: BOLD:ACK4391.

Distribution. This is the first record of the species from Namibia, otherwise recorded from numerous countries: Algeria, Australia, Democratic Republic of the Congo, China, Egypt, Bangladesh, India, Indonesia, Japan, Lebanon, Myanmar, Namibia, Nigeria, South Africa, Spain, Sudan, Uganda and Zimbabwe (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004; Qi et al. 2012).

***Kiefferulus brevipalpis* (Kieffer, 1918)**
Fig. 10E

Material examined. Namibia • 3♂2♀; OTJOZONDJUPA; Gross Barmen; 22°6'38.16"S, 16°44'42"E; 4 Dec. 2018; X. Lin leg.; sweep net; SHOU; BOLD specimen code: NAM-Chiro46; NAM67; NAM64; NAM63; NAM62; BOLD sequence ID: NAMOE046-22; NAMCH057-20; NAMCH054-20; NAMCH053-20; NAMCH052-20; BOLD BIN: BOLD:AFG1076; BOLD:ACK6259 • 7♂; KHOMAS; Windhoek; 22°36'43.2"S, 17°5'27.6"E; 3 Dec. 2018; X. Lin leg.; light trap; SHOU; BOLD specimen code: NAM-Chiro22; BOLD sequence ID: NAMOE022-22; BOLD BIN: BOLD:AFG1076.

Distribution. This is the first record of the species from Namibia, otherwise being recorded from Democratic Republic of the Congo, Ethiopia and Uganda, (as. *Nilodorum brevipalpis* (Kieffer, 1918)) (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Martin 1999).

***Parachironomus acutus* (Goetghebuer, 1936)**
Fig. 10F

Material examined. Namibia • 1♂; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW.

Distribution. This is the first record of the species from Namibia, otherwise species is recorded from Cameroon, Chad, Democratic Republic of the Congo, Madagascar, Nigeria and South Africa (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004). No specimens of this species were successfully barcoded as a part of this project. This species is often treated as "unplaced" within Chironomini (Freeman and Cranston 1980), we are following Ekrem et al. (2017) in placing this species in *Parachironomus*.

***Paracladopelma rhodesianus* (Kieffer, 1923)**
Fig. 10G

Material examined. Namibia • 1♂; KHOMAS; Windhoek, Arebbusch river; 22°34'28.92"S, 17°3'15.84"E; 3 Dec. 2018; X. Lin leg.; sweep net; SHOU.

Distribution. This is the first record of the species from Namibia; the species was otherwise recorded from Botswana, South Africa and Zimbabwe (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004). No specimens of this species were successfully barcoded as a part of this project.

***Polypedilum abyssiniae* Kieffer, 1918**
Fig. 10H

Material examined. NAMIBIA: • 1♂; OTJOZONDJUPA; Von Bach Dam Nature Reserve, Swakop river outflow; 22°0'53.28"S, 16°57'12.24"E; 2–10 Dec. 2018; V. Baranov leg.; Malaise trap; NMNW.

Distribution. The species was previously recorded from Namibia (Kunene river) (Harrison 2004), otherwise it was recorded from Chad, Democratic Republic of the Congo, Ethiopia, Mali, South Africa, Sudan and Tanzania (Freeman 1956; Freeman and Cranston 1980; Curtis 1991; Harrison 2004). No specimens of this species were successfully barcoded as a part of this project.

Key to the *P. dewulfi* - species group sensu Sæther and Wang (1995) from the Afrotropical Region

1 Anal point with bare, spatulate tip, cell m proximal to *r–m* with 4–77 setae (widely distributed in Nearctic and Palaearctic, first Afrotropical record listed here, see below) ..... ***P. impensus* (Walker)**
– Anal point with bare tip which might be parallel-sided (narrow or wider), or pointed, cell *m* proximal to *r–m* with 0–70 setae..... **2**
2 Bare apical part of the anal point long and narrow, cell *m* proximal to *r–m* with 0–7 setae (South Africa, D.R. Congo, Saudi Arabia, South Africa, Tanzania, Zimbabwe) ..... ***P. dewulfi* (Goethgebuer)**
– Bare apical part of the anal point otherwise, cell *m* proximal to *r–m* with 10–70 setae..... **3**
3 Anal point with a narrow (2–5 µm) wide apex, completely devoid of setae; wing cuneiform..... ***P. cuneipennis* (Freeman)**
– Anal point with a wider apex, bearing a few setae; wing not cuneiform.... **4**
4 Apex of anal point very wide (16 µm), crista dorsalis of gonostylus is of similar height during its entire length..... ***P. crassicaudatus* Sæther & Wang**
– Apex of anal point 5 µm, crista dorsalis forms a prominent triangular protrusion at the distal end of the gonostylus ..... ***P. namibiae* sp. nov.**

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

Specimens were collected from the Khomas, Otjozondjupa and Hardap regions of central Namibia between 27 Nov. – 8 Dec. 2018, under the collective research permit issued by NCRST (authorization number AN20181007). All insect material was then exported to Germany for processing under an export permit issued by the Ministry of Environment and Tourism of Namibia (Number 119666).

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Author contributions

VB have conceptualised the study, obtained funding, have conducted morphological morphological identification of the specimens, have conducted the imaging and data analysis, and written a first draft.; VB and XL have conducted sampling; XL, CC and JH have conducted DNA barcoding lab work; All authors have worked on the 1st and 2nd drafts.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Specimens/Taxa group A (core/source species)

Authors: Viktor Baranov, Xiaolong Lin, Jeremy Hübner, Caroline Chimeno

Data type: xlsx

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Link: https://doi.org/10.3897/AfrInvertebr.65.111920.suppl1

Supplementary material 2

Tree Result - DS-NAMCHIR (116 records selected)

Authors: Viktor Baranov, Xiaolong Lin, Jeremy Hübner, Caroline Chimeno

Data type: pdf

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Supplementary material 3

COI FULL DATABASE includes records without species designati...

Authors: Viktor Baranov, Xiaolong Lin, Jeremy Hübner, Caroline Chimeno

Data type: pdf

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